Vaccination and Atherosclerosis

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Atherosclerosis is an inflammatory disease. Both innate and adaptive immunity are involved in lesion formation and development. A number of antigen candidates, such as oxidized low-density lipoprotein and heat shock protein, have been associated with the inflammation and immune reaction that is part of the atherosclerotic process. Because experimental models of some other inflammatory/autoimmune diseases can be improved by vaccination, it is of interest to investigate if vaccination can also be applied to prevent or retard atherosclerosis. Indeed, the modification of immune responses in animal models can greatly affect the development and progression of atherosclerosis. This review provides an overview of our current understanding of effects and proposed mechanisms of immunization on preventing atherosclerosis.

Introduction

Vaccination originally referred to a phenomenon in which the infection with a bovine analogue of smallpox, vaccinia, stimulated an immune response that cross-reacts with smallpox and thereby conferred protection from the human form of the disease. The term has been further extended to the induction of immunoprotection to other infectious agents. Vaccination has been a very effective way of controlling infectious diseases over the past 200 years, virtually eliminating several human lethal diseases such as diphtheria, polio, and measles in the Western world.

Atherosclerosis is characterized by patchy subintimal thickenings of medium and large arteries, which reduce or obstruct blood flow and lead to myocardial infarction, cerebral infarction, aortic aneurysm, and peripheral vascular diseases. As a consequence, atherosclerosis remains the principal cause of death in the Western world and much of Asia. In recent years, the pathogenesis of atherosclerosis has been revealed to be related not only to cholesterol deposition, macrophage infiltration, and smooth muscle cell (SMC) proliferation in the lesions, but also systemic and local innate and adaptive immune responses [1••,2••].

The first line of immune defense is dependent on detection of pathogen-associated molecular patterns (PAMPs), which evoke an inflammatory response. These PAMPs include endotoxin/lipopolysaccharides (LPS), lipoteichoic acid, heat shock proteins (HSPs), peptide glycans, and prokaryotic DNA motifs, which can all be recognized by the scavenger and Toll-like receptors on macrophages. Such a ligation leads to endocytosis and lysosomal degradation of the PAMP-coated particles and activates nuclear factor κB (NF-κB) signaling pathway in phagocytes [3]. Importantly, pattern recognition receptors, including scavenger receptors (SRs) and probably also Toll-like receptors (TLRs), bind oxidatively modified low-density lipoprotein (LDL) particles [4–6]. LDL modification, therefore, generates PAMP and elicits innate immune responses. Studies have further indicated that the initiation of atherosclerosis can be viewed as a response of the innate immune system to the accumulation and modification of lipoprotein in the intima. Indeed, lack of scavenger receptor A (SR-A) or CD36 through gene-knockout technique in vivo decreases the development of atherosclerosis in murine models [7,8].

T cells, which are key representatives of adaptive immunity, also participate in the formation of atherosclerosis as early as monocytes and macrophages [9•]. Atherosclerotic lesions contain significant amounts of activated T cells, with the expression of T-cell cytokines such as interferon γ (IFNγ) and of cytokine-induced genes like HLA-DR in the lesions [10,11]. The expression of HLA-DR by activated macrophages and activated T cells adjacent to these macrophages in the lesions strongly suggests that a cell-mediated immune reaction is taking place in the process of atherosclerosis. Lack of IFNγ or its receptor as well as complete lack of adaptive immunity in murine models leads to less lesion formation [12–15], whereas T-cell transfer or direct IFNγ injection exaggerates atherosclerosis [13,16].

Although a minority of lymphocytes in human plaques bears markers for B cells, mRNA expression of κ-chain was detected in the lesions of animal model [17]. High-titer autoantibodies, produced by activated B cells and specific to oxidized (ox) LDL, have been found in circulation and lesions of humans and hypercholesterolemic animal models [18,19]. Furthermore, complement factors such as C1 and C3b, terminal C5b-9 complement complex, and complement receptors have also been found in human and animal lesions. These findings suggest that activation of humoral immune responses is also involved in the disease process [20,21]. The findings that removal of B cells via splectomy enhances lesion formation whereas polyclonal

intravenous immunoglobulin (IvIg) injection suppresses the disease are in agreement with this hypothesis [22,23•].

It has been shown in animal models that some inflammatory and autoimmune diseases such as rheumatoid arthritis and multiple sclerosis could be attacked by means of vaccination [24,25]. It is, therefore, of particular interest to explore if vaccination can be introduced to prevent or retard atherosclerosis, given the results that the manipulation of immune response in animals can greatly affect the development and progression of atherosclerosis [12,13,26–29]. Indeed, studies on this issue have provided promising data over the past few years. This review discusses several potential antigens that have been found to be involved in the development of atherosclerosis, with specific focus on the effect and proposed mechanism(s) of immunization.

Antigens and Immunization in Atherosclerosis

Oxidized low-density lipoprotein

An increased level of serum cholesterol, especially LDL cholesterol, is a well-known risk factor for atherosclerosis [6]. LDL can be oxidized in the intima of the artery wall; this may be mediated by endothelial cells (EC), SMCs, and macrophages via several pathways, including those involving superoxide anion, ceruloplasmin, and lipoxygenase. OxLDL, one of the most important autoantigens, is, therefore, present in atherosclerotic lesions [30]. Macrophages can present neoantigens such as oxLDL to induce a T-cell–dependent immune response [31]. Indeed, SR-mediated uptake of oxLDL can lead to processing and presentation of particle fragments as major histocompatibility complex (MHC) class II bound antigens [31]. This process elicits a CD4+ T-cell response. As a matter of fact, 10% of CD4+ T cells cloned from human lesions recognized oxLDL [32]. It is likely that many of these T cells were activated by oxLDL presented in regional lymph nodes, but direct antigen presentation in the plaque remains an additional important mechanism for activation of oxLDL-specific CD4+ T cells.

High-titer autoantibodies, produced by activated B cells and specific to oxLDL, have been found in blood and lesions of humans and hypercholesterolemic animal models [18,19,33]. The titer of autoantibodies to epitopes of oxLDL has been correlated with the extent of atherosclerosis in humans and murine models [18]. However, another study failed to show such a correlation in humans [34].

Apart from eliciting adaptive immunity, intimal oxLDL activates macrophages and ECs to express adhesion molecules, recruits immune cells and SMCs to the arterial intima, transforms macrophages into foam cells, impairs nitric oxide activity, induces complement activation, and impacts on coagulation and thrombosis [35–38]. OxLDL also induces secretion by macrophages, SMCs, and antigen-specific T cells of tumor necrosis factor α (TNF-α), interleukin (IL)-1, -8, -12, -15, and IFNγ; all of these cytokines may contribute to the development of atherosclerosis [32,39–42]. High concentrations of oxLDL exhibit cytotoxic effects on ECs and SMCs, suggesting that they may cause endothelial dysfunction and promote fatty streak evolution [43].

Immunization with oxLDL ameliorates atherosclerosis in several animal models [44,45]. This effect is associated with increased titer of IgG anti-oxLDL (*ie*, T-cell– dependent B cell response) [45] and may, therefore, involve T-cell as well as B cell activation. Immunization with oxLDL also leads to a reduction of neointimal formation after balloon injury [46]. Such protective effects might involve Fc-dependent removal of oxLDL from the circulation or neutralizing the effects of oxLDL systemically or locally. It may also relate to the activation of cellular immune responses [45].

As LDL is a complex lipoprotein particle with molecular weight of approximately 2000 kD, it is necessary to define the antigenic epitopes in order to pursue vaccine development. In recent study, immunizing mice with human apolipoprotein (apo)B100 peptides led to a reduction in lesion size [47•]. Two groups of peptide mixture were chosen. The first group consisted of two peptides of the human apoB100 with 85% to 90% homology to mice, whereas the second mixture contained five peptides, three of which were completely nonhomologous to mice. In the first immunization group, there was an approximately 60% decrease in lesion size accompanied by increased collagen in plaques and elevated titers of IgG antibodies against oxLDL in the circulation. However, the other group did not show any protective effect on the development of atherosclerosis [47•]. Protein sequence, therefore, appears to play a crucial role for the immunoprotective effect.

It seems conflicting that immunization-induced humoral immune response to oxLDL is protective [44,45], whereas high titer of autoantibodies against oxLDL is correlated with the extent of atherosclerosis [18]. Such a contradiction may be explained by a hypothesis that there are two different types of antibodies produced in vivo. One is a "protective" antibody induced by immunization and related to the clearance of oxLDL cholesterol from circulation, whereas the other is a "pathogenic" autoantibody involved in the deposition of cholesterol in the arterial wall [48]. Alternatively, antibodies may reflect immunization, but neither play any important pathogenetic role nor protect from the disease. If this is the case, cellular immune responses may be decisive. Immunization experiments in immunodeficient mice should shed light on these possibilities.

Heat shock protein

Heat shock proteins, another group of proposed atherosclerosis-related antigens, may also contribute to autoimmunity in the disease [49•]. HSPs, which are expressed ubiquitously and constitutively and function to stabilize and protect newly synthesized proteins during folding, are released by injured cells and by monocytes exposed to oxLDL [50]. HSPs can serve as targets for autoimmune responses in many inflammatory diseases such as Crohn's disease and rheumatoid arthritis. HSPs display striking homologies throughout

evolution, with significant sequence similarity between mammalian HSP60, chlamydial HSP60, and mycobacterial HSP65 [51]. It is, therefore, possible that some immune reactions to HSPs reflect molecular mimicry between microbial and human antigens [49•]. Several types of HSPs have been found in atherosclerotic lesions [52,53]. The titer of antibody to HSP65 appears to correlate with the progression of atherosclerosis [54]. Circulating antibodies to HSP65 in patients with atherosclerosis may reflect an autoimmune reaction to injured endothelium, which expresses high levels of HSP65. It is likely that this autoimmune response is not the primary event in atherosclerosis but secondary to an initial assault. An alternative and equally possible explanation could be that these antibodies may initially bind to HSPs on invading microbes and then cross-react with their human homologue HSP60, which may account for the association between infections and atherosclerosis [51].

Immunization with HSP60 has been reported to aggravate fatty streak lesion formation in atherosclerotic rabbit and mouse models [53,55]. Such an immunization induces vascular inflammation with infiltrates of HSP-reactive T cells [53]. The mechanisms involve both cellular and humoral immune reactions because adoptive transfer of HSP-specific lymph node cells or repeated intraperitoneal administration of IgG from serum of HSP-immunized mice promotes fatty streak formation [56]. Interestingly, HSP60, like bacterial endotoxin, can also activate Toll-like receptor 4 on mononuclear cells in a CD14-dependent manner [57], which suggests that HSPs not only induce adaptive immune responses but activate innate immunity as well.

Interestingly, a recent study shows an atheroprotective effect of HSP by mucosal immunization [58•]. Maron *et al.* [58•] applied nasal administration of HSP-65 on LDL-receptor knockout (LDLR KO) mice, and found that such a treatment significantly attenuated atherosclerotic lesion formation and T-cell infiltration accompanied by elevation of IL-10 expression in the lesions and of Th2-driven IgG1 anti-HSP antibodies in blood. The mechanisms of this atheroprotection may involve specific immune tolerance formation due to suppression of proinflammatory Th1-type immune response and induction of IL-4–secreting Th2 cell formation, as well as enhancement of anti-inflammatory cytokine IL-10/transforming growth factor-β expression. Such effects are known to develop in response to mucosal immunization [58•,59].

Microbial antigens

Seroepidemiologic studies during the past 15 years show an association between cardiovascular diseases and microbial infections with certain bacteria as well as viruses of the herpes family. *Chlamydia pneumoniae* has been considered to be the most common microbe associated with cardiovascular diseases. Patients with acute myocardial infarction have high titers of antibodies against *C. pneumoniae* [60]. Interestingly, *C. pneumoniae* can be found in atherosclerotic plaques [61]. One study has reported a significant relationship between the abundance of *C. pneumoniae* and the severity of atherosclerotic disease [62], but others failed to detect a similar association [63,64].

In animal studies, rabbits infected with *C. pneumoniae* display increased vascular inflammation and may also develop larger atherosclerotic lesions [65]. However, findings are contradictory in murine models. Some investigators report that infection of mice with *C. pneumoniae* accelerates atherosclerosis [66], whereas others could not detect any such correlation [67,68]. Therefore, the relationship between infection with *C. pneumoniae* and disease development needs to be further clarified.

Infection with *C. pneumoniae* might affect atherosclerosis by secreting LPS to induce acute inflammation with C-reactive protein elevation, or by releasing HSP60 to cause a chronic reaction that induces autoimmunity against vascular cells. Some studies suggest that antibiotic treatment can attenuate disease progression concomitant with increased titers of antibodies to *C. pneumoniae* [69,70]. However, the Weekly Intervention with Zithromax for Atherosclerosis and Its Related Disorders (WIZARD) study [71•], the largest trial of antibiotic therapy for coronary artery disease, did not detect any beneficial effect of azithromycin on myocardial infarction. This argues against *C. pneumoniae* as a cause of coronary heart disease [71•].

Phosphorylcholine, one of the structural components of bacteria, is also present in oxLDL [72]. Some autoantibodies reactive against oxLDL cross-react with phosphorylcholine and protect against common pneumococcal infections [33,73]. Recently, Binder *et al.* [74••] showed that immunization of LDLR KO mice with *Streptococcus pneumoniae* dramatically reduced lesion development in parallel with elevation of oxLDL-specific IgM antibodies that cross-react with pneumococcal epitopes. This study suggests a new potential vaccination-based strategy for ameliorating atherosclerosis.

The herpes group viruses, including cytomegalovirus (CMV) and herpes simplex virus type I (HSV-I), have been associated with atherosclerosis [75]. Both CMV and HSV are found in atherosclerotic lesions. CMV is also linked to transplant arteriosclerosis and restenosis after coronary atherectomy [76,77]. Infection of apoE knockout (apoE KO) mice with murine γ-herpes viruses and CMV aggravates atherosclerosis [78,79]. The role for these viruses in disease progression may be due to their effects on smooth muscle cell migration [80].

Another virus, influenza, has also been suggested to cause increased cardiovascular disease and to trigger myocardial infarction [81,82]. Vaccination against influenza in humans is associated with reduced risk of recurrent myocardial infarction [83]. Recently, Naghavi *et al.* [84] have reported that infection of apoE KO mice with influenza A virus significantly increases atherosclerosis, suggesting that influenza A virus may have proatherogenic effects.

However, the fact that atherosclerosis in germ-free apoE KO mice is not different from that in apoE KO mice raised with ambient levels of microbial challenge suggests that

infectious agents are not necessary for atherosclerosis [85]. Instead, they may play an important accelerating role in the disease progression.

Beta 2-glycoprotein and others

The β2-glycoprotein I (β2-GPI) is present on platelets and on the surface of ECs. Autoantibodies against β2-GPI are associated with prothrombotic effects and produced in patients with antiphospholipid syndrome as well as other inflammatory diseases, including atherosclerosis. β2-GPI is also found in the subendothelial regions and the intima of human atherosclerotic plaques [86]. High titers of anti–β2-GPI antibodies together with anti-oxLDL antibodies are observed in the patients with coronary artery disease [87]. Immunization of LDLR KO mice with human β2-GPI remarkably accelerates atherosclerosis [88]. Such a proatherogenic effect of anti–β2-GPI immunity may be due to the fact that anti–β2-GPI antibodies can activate ECs [88] and enhance the uptake of oxLDL by macrophages [89].

Another glycoprotein related to atherosclerosis is cholesteryl ester transfer protein (CETP), which is secreted from the liver and bound mainly to high-density lipoprotein (HDL). The function of CETP is to transfer cholesteryl esters from HDL to LDL and very low-density lipoprotein (VLDL). The effect of CETP on atherosclerosis is controversial; it is either antiatherogenic when CETP increases the rate of reverse cholesterol transport from peripheral tissues to the liver for elimination, or proatherogenic when CETP redistributes cholesteryl esters from HDL to LDL/VLDL and/or decreases the HDL concentration in peripheral tissues [90]. Mice transgenic with either human apoC-III or human lecithin-cholesteryl acyltransferase exhibit increased atherosclerosis [91,92], whereas introduction of CETP into these mice ameliorates the disease [91,93]. However, overexpression of CETP in apoE KO mice and LDLR KO mice accelerates atherosclerosis [94]. Rittershaus *et al.* [95] have recently reported that immunization of cholesterol-fed rabbits with CETP attenuates disease progression accompanied by increased levels of HDL cholesterol. The mechanism of such an atheroprotection may be a suppression of CETP activity by vaccination-induced antibodies.

The role of platelet-derived growth factor (PDGF) in atherosclerosis has also been tested by vaccination. PDGF is an important growth factor that may mediate migration and proliferation of SMCs in the intima [43]. It can be secreted by dysfunctional ECs, monophages, and macrophages, as well as by SMCs in lesions. Immunization of cholesterol-fed rabbits with recombinant human PDGF significantly reduces lesion formation in parallel with raised antibodies against PDGF [96]. Such a protection may be due to the specific antibodies neutralizing atherosclerotic effects of PDGF [96].

Conclusions

Many studies suggest that modification of the immune response could affect the process of atherosclerosis, given the evidence that vaccination with certain antigens attenuates the disease. The results from immunization of animals with oxLDL or certain peptide sequences derived from oxLDL are encouraging for the future development of a vaccination approach in atherosclerosis prevention and treatment. Although the microbial theory in the pathogenesis of atherosclerosis remains unproved, mucosal HSP vaccination may provide a new immunologic insight into the treatment of the disease. The antiphosphorylcholine studies have opened a new window to the molecular mimicry between certain bacterial infection and LDL oxidation. It will, therefore, be interesting to see if transfer of such an antiphosphorylcholine specific antibody can protect from atherosclerosis.

It is clear that much work will be required to locate the precise molecular epitopes of the atherosclerosis-related antigens and to clarify the mechanism involved in atheroprotection achieved by immunization. At present, we are still far from a clinical application in patients with atherosclerosis and its associated complications. However, recent results obtained in experimental models encourage further research in this field.

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